BOTANY.

New Studies in Fecundation.—M. Leon Guignard publishes a paper in the[1] *Annales des Sciences Naturelles*, entitled "New Studies of Fecundation, with a comparison of the morphological phenomena observed in plants and in animals."

He reminds us that until very recently the essence of fecundation was supposed to reside exclusively in the union of two nuclei of different sexual origin, and that the protoplasm played but an accessory part in the process; that in phenogamous plants, for instance, the male nucleus penetrates alone the oosphere to the exclusion of its accompanying protoplasm. His recent observations, however, and the discovery of certain new bodies, new at least in vegetable cells, which he calls "directive spheres" and which play their part in the process throw quite a new light upon the subject and moreover bring into close accord the phenomena as existing in the two kingdoms. He follows the development of the reproductive elements, male and female, from the very beginning to their adult stage so as to determine the mode of differentiation by which they acquire sexual characters and then further follows these sexual elements, showing which are the essentially active parts, and how the union between them takes place to form the first embryonic cell.

The account which he gives of the technique employed in his researches is far from satisfactory; indeed he says himself that the "technique applicable to the study of vegetable protoplasm leaves much to be desired." As fixative agents he used absolute alcohol, corrosive sublimate, picric acid and osmic acid; to stain the protoplasm and the spheres he hardened the specimen in absolute alcohol and then in 10 per cent. solution of zinc sulphate or ammonia alum, followed by hæmatoxylin. Fuchsine and methyl green proved also of special value in distinguishing the plasmic contents of the cells.

He first describes the formation of the reproductive cells, the pollen grains and the embryo sac, using as his subject a species of lily, *Lilium martagon*. He traces each step in clear language and by the aid of beautiful figures, from the undifferentiated meristem of the budding anther to the archesporia or pollen mother-cells, to the special mother-cells and the mature pollen grains; and with the same nicety, the origin of the embryo sac with the egg apparatus (oosphere

[1]Ann. Sci. Nat.; Bot. Tome XIV. pp. 163–288. Plates 9 to 18.

and synergidæ), the antipodal cells and the secondary nucleus. In all this there is nothing essentially different to what may be found in the better text-books. But at a certain point in the development of the pollen, he shows us cells in stages of karyokinesis having each just twenty-four chromatic rods, each rod with its parallel halves stuck together throughout their length but destined to separate and form parts of the two new nuclei. In subsequent figures are shown the well formed anthers, the completed pollen sac and the special mother-cells. Upon the completion of the tapetal layer the primary mother-cells cease to divide and become the special mother-cells which now increase in size, become filled with protoplasm, each with a large nucleus and several nucleoli and closely appressed to the nuclear membrane, the two directive spheres lying side by side. Within the nucleus is a band of chromatin which may be likened to a tangle of endless narrow ribbons. In the last division by which these cells were formed the nuclei still showed the twenty-four rods of chromatin. There now follows a resting stage preparatory to forming the pollen grains. As the nucleus is to enter into activity its chromatic frame-work (charpente) may at least in part be traced out along its very sinuous course but without discovering any free ends within the nuclear cavity or any evidence of its being aught than a continuous endless filament; which means that the twenty-four rods must have fused end to end in its formation. A little later are to be seen twelve segments, each formed of two series of chromatin granules, free in the nuclear fluid. The halvès of these rods are exactly the same in length and are united by a common hyaloplasm and from their ends also are still projecting fine delicate threads of the same plasma. The directive spheres now take their place as poles, the rods are carried to the equator and the spindle is soon completed. The halves of the rods now separate, that is, the ends directed towards the center or axis of the spindle, part first and the segments on their way back to the poles assume the forms of the letters U or V. The two nuclei thus formed at once enter again into division and the four pollen grains are perfected.

With each nuclear division the "directive spheres" have doubled, so that each new nucleus is provided with two spheres.

For the formation of the generative or sexual nucleus proper, the pollen nucleus divides to form a vegetative and generative nucleus.[1] Within the pollen tube the generative nucleus undergoes a second division, the spheres likewise doubling, and, as the plane of division is

[1]By the artificial culture of pollen the generative nucleus sometimes quite escapes from the pollen tube and offers a most favorable view of the directive spheres.

transverse to the length of the tube, the two new nuclei are so placed that the lower one is preceded, and the upper one followed, by its two spheres. Both of these nuclei are accompanied by their protoplasm and each has been seen to have its twelve chromatin rods. If the vegetative nucleus has not already disappeared, it may be distinguished from the sexual elements by the difference in reaction to methyl green and fuchsine.

The steps by which the differentiation of the female factor arises may be rapidly sketched as follows: A large cell in the axial row of the nucellus is the mother-cell of the embryo-sac. In all the cell divisions of the nucellus up to the moment when one of the cells becomes conspicuously larger and ceases to undergo further division the nuclei have all shown twenty-four chromatin rods. The embryo-sac grows rapidly so as to come to occupy a large part of the nucellus, sometimes descending towards the center of the same. Its nucleus contains one or more large nucleoli in the meshes of the tangled ribbon made up of two series of chromatin granules. The two directive spheres lie in contact with the nuclear membrane and the cytoplasm is now seen radiating without regard to the position of these spheres. The ribbon now breaks into rods and the substance of the nucleoli so changes as no longer to be distinguishable from that of the chromatin segments, the number of which is now twelve not twenty-four. The spheres become active, go to the poles and are surrounded by cytoplasmic rays. At first the rods are scattered in a disorderly manner through the forming spindle, but are soon carried and properly oriented by the achromatic threads about the equator so that there may now be seen a rod to each large, thread, there being however some more delicate intermediate threads to which no rods are attached. Thus is formed the spindle and disk. The rods are easily seen to consist of longitudinal halves and in the further change they split at the ends directed towards the spindle axis, one-half appearing to glide up a thread towards one pole, the other half moving down towards the opposite pole until they finally part and become new groups of twelve contorted segments, shaping themselves into two new nuclei. The centrosomes in the meantime have doubled, forming two spheres at each end of the old nuclear axis. The new nuclei so formed appear in all respects quite similar and they move to opposite ends of the embryo-sac. The lower one especially gains in size, and both continue to divide. But in the next division the lower one is seen to have not twelve, but sixteen or twenty rods, whilst the upper one and its derivatives still have but twelve. There are now four cells or nuclei above

and four below; two synergidæ, the oosphere and a polar cell above, three antipodal cells and a polar cell below. As the oosphere has but twelve rods, the two sexual cells have thus the same number of these elements. The upper polar nucleus becomes larger than its brother nucleus of the oosphere. The lower polar nucleus moves round a large vacuole and joins the upper one of the same name, while the antipodal cells disappear. The spheres of the synergidæ are situated right and left, because in the division by which they were formed the spindle axis was horizontal; whilst the spheres of the oosphere and upper polar nucleus are in couplets above and below these nuclei because the spindle was vertical. This position of the spheres is of importance in the act of copulation, for the first step in this process is the meeting of the two spheres from each copulating nucleus and as the male nucleus is moving down the pollen tube with its two spheres head on, so the oosphere must so present its spheres as to squarely meet them; and to the same end the spheres of the approaching polar nuclei are most conveniently situated.

The approach of the sexual nuclei is as follow: The vegetative nucleus has disappeared while going along the pollen tube. The tube becomes narrow and insinuates its end between the epidermic cells of the nucellus. Arrived at the summit of the sac it enlarges into a flask-shaped extremity and pushes the membrane of the sac before it as it continues to advance inward either in a straight course or obliquely, without however spreading out its end upon the sac as is the case in *Monotropa* and in *Orchis latifolia*. From this time the membrane of the sac and of the tube are not to be distinguished. The tube enters by the side of the synergidæ or directly into one of them, in which latter case the contents of the synergidæ assume the peculiar appearance of disorganization. Strasburger thought that this was the rule and that the office of the synergidæ was to transmit the contents of the pollen tube to the oosphere. Whatever be the method of penetration, the male nucleus which arrives first in contact with the embryo-sac passes through the membrane and rapidly joins the nucleus of the oosphere. Strasburger now thinks that the liquid which directs this amorous flight of the male to the female nucleus is furnished by the synergidæ, playing the part which is attributed to malic acid and malates in directing the course of the antherozoids in the cryptogams. Guignard believes that this attraction may reside simply in the protoplasm of the female cell.

The male nucleus, as has been shown, is preceded by its two spheres which lie side by side and which are thus brought face to face with the

spheres of the female nucleus which they quickly join and so form two new couplets of spheres, each couplet containing a sphere of two different elements. This conjunction of the spheres takes place before the nuclei unite, but they do not fuse until the division of the nucleus begins. While still as couplets they take their position above and below, marking out the axis of the coming spindle, which is that of the long axis of the oospore. The second male nucleus sometimes escapes from and sometimes remains in the pollen tube and ultimately disappears in the protoplasm. The reason for the existence of two male nuclei is in doubt. That the division of the single primary one into the two smaller masses is so that the male and female nuclei may be nearly in equilibrum as to size, is not a satisfactory suggestion; and since the two male nuclei are equally active and effective in fertilization, the division of the primitive nucleus could not have been a matter of differentiation. After a long contact of the male and female nuclei, the nuclear membranes at the surface of contact disappear and the contents form a common mass; that is, the nuclear fluids mingle, but there is not a true fusion of the chromatic elements. Contraction and orientation of these elements follow with the formation of a spindle and nuclear disk, and a return to twenty-four rods. Twelve of these may have been furnished by each nucleus but they are not to be distinguished from one another. The two groups of secondary segments collect at the poles, a wall of cellulose is formed at the equator and two embryonic cells come into existence. Thus fecundation has resulted in doubling the number of rods in the first segmenting nucleus.

It happens with curious constancy that the secondary nucleus of the embryo-sac begins its division for the formation of endosperm, just at the time that the male nucleus penetrates the oosphere. This secondary nucleus formed by the union of the two polar nuclei, which were larger and rich in chromatin, contains a greater number of rods, as do its derivatives, than are to be found in the egg nucleus. So likewise do all the nuclei of the endosperm contain a greater number of rods than do the nuclei of the rest of the ovule and other organs of the plant. This variation in the number of rods opposes the idea of the chromatic segments retaining their individuality during the resting stage of the nucleus. In *Leuconium* and in *Galanthus*, as the embryo-sac becomes somewhat crowded with nuclei and their spheres, and as cell walls arise, there may be included as many as ten or twelve nuclei in a single cell. Under these conditions the spheres act upon closely neighboring nuclei in such a manner as to form multipolar spindles. Such figures have lately been seen by Henneguy in the parablast of

the trout, a tissue comparable to the endosperm, and also in epithelial tumors. From such multipolar figures more than one daughter nucleus may naturally be expected to be formed.

Most of these observations have been taken from the lily. Other plants show differences of more or less moment; thus, it is found that the number of chromatin rods, so constant in the sexual cells for the species, may yet vary in members of the same family.

Guignard's work was awarded the Bordin prize by the Academy of Sciences of Paris. The title of the subject given for contest required an exposition of the relations which exist between the phenomena attending fecundation in the vegetable kingdom, with those observed in the animal kingdom. This obliged him to summarize the work done by zoologists in this field, and there follow at this point some twenty pages devoted to the consideration of the sexual cells in animals, the polar globules (richtungskörper), their homologies with the spermatozoids and their general morphological value. In all this he rests upon the contributions of Lowen, the Hertwigs, Fol, Van Beneden, Boveri, Weismann, Ischikawa, Blochman, Bütschli, Whitman and others. The judges, as we learn from another source, say that this part of the work was done with skill and conscientious care. From Guignard himself we get the impression of the strict analogy of the processes of fecundation as they occur in the two kingdoms. Under the heading "General Exposition of Results" the author retraces the subject matter already given: the fixity or constancy of the chromatic rods as to number in the sexual cells; the appearance of and the mode of reduction in the number of these rods at a given stage; the constitution of the nuclei, including a discussion of the individuality of the chromosomes; the existence of "directive spheres" in all vegetable cells; the role or function of these spheres which are distinctive organs of the cells and, lastly, a review of the prevailing theories concerning the phenomena of fecundation. Of this he says, "We see that fecundation is not only a conjugation of nuclei, but accompanying this act is that of the fusion of two protoplasmic bodies whose essential elements are represented in the directive spheres of the male and female cells."

While hovering so closely about the subject of heredity it was not to be expected that it should go untouched. After noticing the theories of several prominent workers in this direction he gives in a single paragraph the gist of his own views by saying, "That all the cells, or at least, a greater part of the cells of the body possess in a latent condition all the hereditary properties of the species. A little bit of the

body can reproduce the entire organism. A branch of willow placed in water develops roots at the expense of cells, which thus come to play a part quite different to what was laid out for them in the original plant, a proof, that is, that they already possessed this property from their conception. On the other hand, a severed root may give rise to buds which bear male and female organs; so that sexual cells may be derived directly from the cellular substance of a root. The epidermic cells of a Begonia may produce an entirely new plant just as with cœlenterates, worms and tunicates, new individuals may come from buds or separate parts of the body of these animals. These facts are trite enough," he adds, "but it is well to recall them to show the solid basis of Hertwig's conclusion that, 'the nucleus, by reason of the phenomena it presents during fecundation, is the support or conservator of hereditary properties and that it reappears in the same form and with the same properties in every cell; that it is a substance removed from the grosser metamorphoses of matter by its inclusion in a special vesicle; that by a complex mode of division the daughter nuclei receive portions of the same substance, no differentiation having arisen. Just as Nägeli claims that his hypothetical idioplasm is spread throughout the body like a fine net-work, so according to my theory, every cell of the body encloses in its nucleus the aggregation of hereditary properties derived from the egg, whilst the specific properties of the cell are bound up with the development of the protoplasmic products, consequently every cell possesses the faculty, under appropriate conditions of reproducing the entire organism.'"—B. W. Barton, M. D., Johns Hopkins University, Baltimore, Md.